

One Molecule, Multiple Cancers:

The Devil is in the Details

"Inside the beltway," is a phrase normally reserved for discussions of careers in national politics, referring as it does to the highway that surrounds the Washington D.C. metropolitan area. However, Christina Annunziata, M.D., Ph.D., has also developed her career as a physician-scientist inside the beltway, first as a medical student and resident at Georgetown University and then rising through the ranks of CCR training opportunities to become a tenure-track Investigator. Over the years, Annunziata's responsibilities have involved her in many NCI protocols, but her own research has remained firmly rooted in the family of transcription factors, NF- κ B. As a student with Jeffrey Cossman, M.D., at Georgetown, she studied nuclear factor kappa B (NF- κ B) signaling in Hodgkin's disease. As a Medical Oncology Fellow with Louis Staudt, M.D., Ph.D., in CCR's Metabolism Branch, she studied the role of NF- κ B in multiple myeloma. Now her laboratory investigates the effects of NF- κ B signaling in ovarian cancer. Annunziata believes that understanding the nuances of NF- κ B function in distinct cell types could lead to effective pharmacological interventions for cancer.

It was definitely the opportunity for strong and dedicated research in a clinical environment that drew me to NIH. I met Lou Staudt, when I was still a doctoral student, and it was a natural fit for me to continue my postdoctoral research in his laboratory. NF- κ B is a very interesting family of molecules. It consists of five subunits that form various combinations of dimers capable of coordinating the expression of multiple genes. It is present as an inactive form in the cytoplasm of most cells, where a variety of external cellular signals can prompt its rapid

separation from a molecular complex and migration to the nucleus to modulate gene expression. NF- κ B signaling is important for many different cell types, however, the pathway functions differently according to cell type.

In multiple myeloma, we found that NF- κ B signaling was turned on in most of the tumors we studied. In many cases, that may have been the result of influences from the surrounding tissue—the tumor microenvironment—but in some cases, the tumors had autonomous aberrant NF- κ B activity. However,

we didn't identify just one specific mutation. We found that there were multiple points throughout the pathway that were dysregulated in different myeloma subtypes. In one case, a protein might be amplified, in another case a negative regulator might be lost. We did these analyses by first looking at changes in gene expression, but in many cases, validated our findings by looking at protein levels. We believe that one therapeutic action of the protease inhibitor, bortezomib, now approved for the treatment of multiple myeloma, may be to inhibit NF- κ B signaling.



Christina Annunziata, M.D., Ph.D.

NF- κ B Signaling in Ovarian Cancer

While working in the Staudt laboratory, I was performing clinical duties one day per week with Elise Kohn, M.D., in CCR's Medical Oncology Branch (See "Ovarian Cancer: A Silent Killer "Speaks" through Proteins," *CCR connections* Vol. 2, No. 2). I began seeing patients with ovarian cancer,

and I began to wonder whether NF- κ B signaling might also play a role in this disease. I could not find much in the scientific literature that addressed this question, and I wanted to move my research more towards helping the patients I was actually seeing. So, when I began my research program as an Assistant Clinical Investigator, I began to study NF- κ B signaling in ovarian

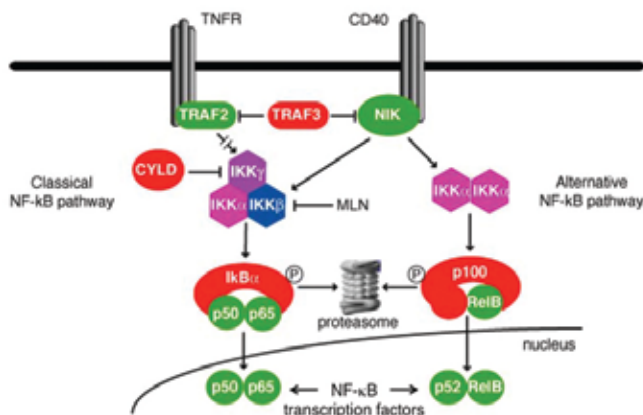
cancer (See "CCR's Clinical Investigator Development Program," Page 31).

My laboratory used a small-molecule drug and small interfering-RNA molecules (siRNA) to inhibit NF- κ B signaling in ovarian cancer cell lines. We found that we could define a genetic signature that reflected the upregulation of NF- κ B signaling in ovarian cancer and that inhibiting NF- κ B signaling affected both this genetic signature and measures of aggressiveness in disease. The signature included genes associated with proliferation, survival, inflammation, adhesion, invasion, and angiogenesis; i.e., all the hallmarks of cancer.

It turns out that NF- κ B regulates a very different set of genes in ovarian cancer as compared to multiple myeloma cells. That is hardly surprising, given the very different developmental and functional profiles of the two kinds of cells, but it does mean that the NF- κ B activation signature needs to be identified for each cancer type.

We collaborate with Michael Birrer, M.D., Ph.D., who moved from

Schematic of NF- κ B Signaling



The NF- κ B signaling pathway can be activated by a variety of receptors on the surface of cells to activate transcriptional networks in the nucleus. Several signaling components within the cell regulate NF- κ B activity.

Smac, IAP, and Cancer Therapy

Members of the inhibitor of apoptosis protein family (IAP) are exciting targets in cancer research these days. Originally studied for their effects on blocking cell death through inhibition of caspase proteins, IAPs are now known to directly affect multiple cellular processes. Cancer researchers are most interested in the ability of certain IAPs to regulate cell survival and tumorigenesis through activation of NF- κ B signaling.

IAPs appear to protect cancer cells from signals related to inflammation in the tumor microenvironment, for example, tumor necrosis factor α (TNF α). Alterations in IAPs are found associated with many human cancers and are typically associated with poor prognosis, disease progression, and chemoresistance.

Five biotechnology and pharmaceutical companies currently have early stage clinical trials under way for drugs that interfere with IAPs. These drugs are called Smac mimetics, because they operate like cellular Smac molecules to tag IAPs for degradation. Smac mimetics cause the rapid depletion of certain IAPs and show potent anti-tumorigenesis activity in cancer models.

CCR to the Dana Farber/Harvard Cancer Center a few years ago. He has access to a lot of ovarian cancer patient samples, and we have been lucky to work with him to verify that the genetic signature we identified in ovarian cancer cell lines is also found in primary tumors. In fact, we have shown that the NF- κ B signature in primary tumors is associated with poor prognosis.

Finding the Right Drug

There aren't many direct NF- κ B inhibitors available, mainly because of issues related to toxicity. So, we're looking at other points in the pathway that might

be more amenable to therapeutic intervention. I am currently working with an investigational drug from Tetralogic Pharmaceuticals, TL-32711, which mimics the cell signaling molecule, Smac. Under certain conditions, Smac shifts the balance of cell signaling from NF- κ B-related proliferation to controlled cell death by apoptosis. (See "Smac, IAP, and Cancer Therapy"). TL-32711 seems to be more potent and specific than other drugs in its class because of reduced cross-reactivity.

We are now studying the effect of Smac mimetics on ovarian cancer cell lines. I also plan to test this drug in

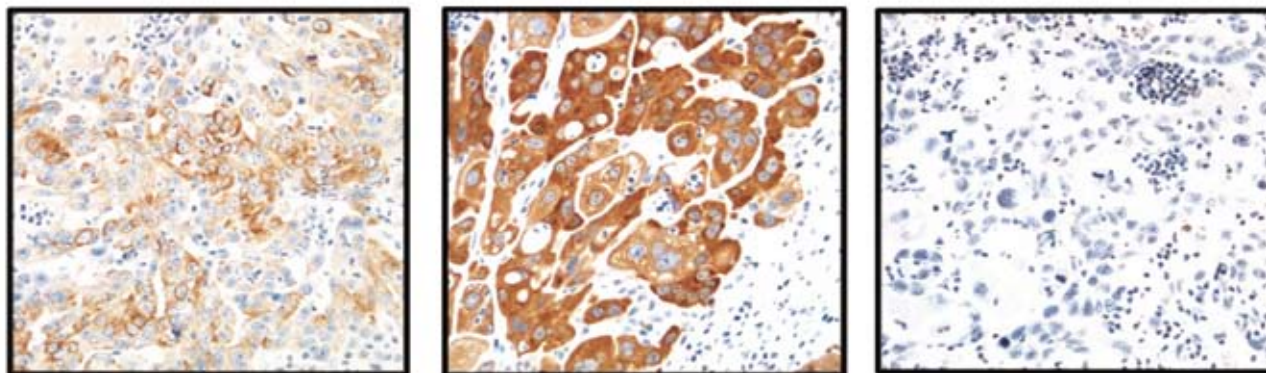
mouse models. NF- κ B is an important part of normal cellular signaling, particularly in the immune system, which is one reason for the toxicities associated with direct inhibitors of NF- κ B. Thus, it will be important to study our Smac mimetic in a mouse model with a normal immune system, so that we can observe the effect of this drug on normal and tumor-infiltrating immune cells. Many mouse models of cancer rely on human cancer cells grafted into a mouse with a deliberately dampened immune system, making such studies impossible.

If, as we predict, Smac mimetics alter expression of the genes we identified as a signature of NF- κ B activity, we will want to move our work into human trials for recurrent ovarian cancer. Although such a trial would not be restricted in its enrollment, our hypothesis would be that patients with the highest levels of NF- κ B activity would be most responsive to the drug. As the trial proceeds, we would look at the treatment response relative to the level of gene expression from an initial biopsy. In that way, we would hope to hone in on the most responsive patient population.

Finding the Right Cancer

I am currently an Investigator on about 10 clinical protocols at CCR, at least half of which are for ovarian cancer. Although my research focus is on NF- κ B signaling, there are several other

(Image: C. Annunziata)



Cells taken from primary ovarian tumors show selective expression of NF- κ B family proteins. Cells stained in blue show expression of IKK- α (left) and IKK- β (middle) stained in brown, but not IKK- ϵ (right).

CCR's Clinical Investigator Development Program

Now a tenure-track Investigator, Christina Annunziata, M.D., Ph.D., came to CCR as a Clinical Fellow in 2002. Subsequently, she and Heidi Kong, M.D., (a recently recruited tenure-track Investigator in CCR's Dermatology Branch) became two of the early participants in the Clinical Investigator Development Program, a 3-year opportunity that serves as a transition between a Fellowship and a position as an independent Principal Investigator. "The program really enabled me to build momentum in my research," said Annunziata.

The goal of the program is to enable promising board-eligible or board-certified translational physician researchers at early stages of their careers to become competitive for tenure-track appointments in academia or comparable positions in government and industry. Program participants are selected via a competitive process and are designated as Assistant Clinical Investigators. Mark Udey, M.D., Ph.D., Chief of the Dermatology Branch and a Deputy Director at CCR, has been instrumental in the program and likens the position to that of an Instructor in traditional academic institutions.

Grant writing skills are emphasized, and participants must apply for an NIH Career Development Award by the end of the second year. The program also includes a component of formal coursework. "This requirement is intended to impart some structure to the program," said Udey, "and we wanted to ensure that our investigators have command of core knowledge central to clinical research."

Applications to the program are accepted annually; up to three positions are available every year. For more information about the Clinical Investigator Development Program, please visit http://ccr.cancer.gov/careers/clinical_programs_invest.asp.

candidate targets for this disease including angiogenesis and poly ADP-ribose polymerases (PARPs). Because some ovarian cancers have dysfunction of *BRCA* 1 or 2, including germline mutations, these ovarian cancers have compromised DNA repair. Inhibiting PARP-associated mechanisms of DNA repair is thought to overwhelm the cell's ability to withstand standard chemotherapeutic agents that disrupt DNA. These were the hot topics at the annual meeting of the American Society of Clinical Oncologists (ASCO) last year, but there are many genetic mutations implicated in this disease, albeit at relatively low frequencies.

A major limitation with all our clinical trials is that we have no way to identify which patients are most likely to respond to a given therapy. Ovarian cancers come in four different histological varieties: serous, clear cell, endometrioid, and mucinous. Interestingly, clear cell ovarian cancer shares some similarity in molecular mutations with clear cell renal cancer and may have a higher response to the drugs sorafenib and sunitinib that are used for renal cancers. So, clear cell

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ovarian cancer may be a molecular subtype that also has a histologic definition. But, that is an exception rather than the rule.

Perhaps even more than in other cancers, it seems that ovarian cancer is extraordinarily heterogenous at a molecular level. For instance, looking at data on genomic instability from The Cancer Genome Atlas (TCGA), you can see distinct hotspots of genetic abnormalities for glioblastoma and lung cancer patients, but so far, you don't see that kind of clustering for cases of ovarian cancer. It might be, therefore, that only five percent of ovarian cancers will respond to a particular drug so, without the appropriate molecular testing, clinical trials will continue to see very low response rates.

Our trials are designed so that patients can stay on the therapy as long as there is a response and side effects are manageable. Patients may, in fact, stay on a drug regimen indefinitely. For

instance, in the case of the angiogenesis inhibitors bevacizumab and sorafenib, we've had several patients treated for two to three years (See "Warrior Drugs," page 32). Although there are side effects including high blood pressure and rashes, this drug combination seems to have tolerable levels of toxicity over time, which patients deem an acceptable impact on their quality of life.

There will likely come a point when the tumor evolves to evade a particular drug, and we will have to switch to another drug or another combination of drugs. So, options are important, which is one reason I continue to pursue my work in NF- κ B. My personal goal, probably like many of my colleagues here at NCI, is to bring my research into the clinic.

To learn more about Dr. Annunziata's research, please visit her CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=annunziata>.